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PRINCIPAL INVESTIGATOR: Coral A. Lamartiniere, Ph.D.

CONTRACTING ORGANIZATION: The University of Alabama at Birmingham
Birmingham, Alabama 35294-0111

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Coral A. Lamartiniere, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of Alabama at Birmingham
Birmingham, Alabama 35294-0111

E-Mail: Coral.Lamartiniere@ccc.uab.edu

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13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

Most soy-breast cancer epidemiological studies conclude that Asian women consuming a traditional diet high in soy products have a low incidence of breast cancer. We have demonstrated that prepubertal exposure to genistein, the primary isoflavone of soy, protects against chemically-induced mammary cancer. The purpose of this work was/is to determine if adult exposure to genistein will protect against chemically-induced mammary cancer and to investigate DNA methylation of estrogen receptor genes as the molecular mechanism of genistein chemoprevention. To date, we have determined that adult only exposure to genistein does not protect against dimethylbenz(a)anthracene-induced mammary cancer. However, prepubertal plus adult exposure to 250 mg genistein/kg AIN-76A diet protected against DMBA-induced mammary cancer. This suggest that exposure to genistein prepubertally may imprint molecular events in the mammary gland that determines the "blue print" from which the mammary cells responds to future hormonal and/or xenobiotic response. In the second year, we will investigate DNA methylation of estrogen receptor genes as the molecular mechanism for genistein imprinting against mammary cancer.

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INTRODUCTION

We have previously demonstrated that short-term exposure of rats to genistein, a soy phytoestrogen, early in postnatal life suppressed chemically-induced mammary cancer in adulthood (1, 2). This novel finding supports the epidemiological reports that Asian women consuming a traditional diet high in soy products have a low incidence of breast cancer (3-5). Furthermore, adjustment for migration rates of Asians to the U.S. revealed that the second, but not the first, generation lose this protection (6). This suggests that exposure to soy early in life confers life-time protection against breast cancer. Since short-term genistein treatment early in postnatal life exerted long-term protection against chemically-induced mammary cancer, we have hypothesized that genistein caused this effect via an imprinting mechanism. For this research, we proposed to investigate 1) the potential of adult genistein treatment to alter susceptibility for breast cancer and 2) DNA methylation of estrogen receptor genes as the molecular mechanism of action.

BODY

Specific Aim 1) To determine the risk of mammary cancer from adult exposure to genistein. This was investigated in the rat-DMBA mammary cancer model. (To be carried out in the first year.)

In the first experiment, we have investigated 2 groups of rats fed AIN-76A diet since parturition, and treated with DMBA on day 50. At day 100 postpartum (shortly after the first tumors can be palpated), we switched one group to 250 mg genistein/kg AIN-76A diet. The results showed no significant difference in tumor formation or adenocarcinoma development (Figure 1). We concluded that 1) rats not exposed to genistein prepubertally do not receive protection from genistein after tumors have developed, 2) genistein does not promote existing mammary tumors, and 3) that genistein exposure must occur prepubertally to exert a chemopreventive effect.

In the second tumorigenesis experiment, we investigated the potential of a combination of prepubertal and adult genistein exposure to protect against DMBA-induced mammary cancer. The purpose of this experiment was to determine if early critical exposure (prepubertal) to genistein would influence how the adult animal would respond to future genistein treatment. Group 1 was fed AIN-76A diet containing 250 mg genistein/kg diet, starting from parturition through day 21 only, and then AIN-76A onward (Gen/DMBA/Zero). Group 2 was fed the genistein diet from parturition through day 21, then AIN-76A only through day 100 postpartum and then from day 100, the genistein-containing diet (Gen/DMBA/Gen). All animals received 80 mg DMBA/kg BW at day 50. As seen in Figure 2, genistein fed to adults already exposed to genistein prepubertally (Gen/DMBA/Gen) had an added level of protection.

Specific Aim 2) To investigate genistein imprinting by methylation of estrogen receptor genes as the mechanism for mammary cancer prevention. This is currently under investigation.

KEY RESEARCH ACCOMPLISHMENTS

1) Dietary genistein given to adult female rats after tumors were initiated, did not alter the multiplicity of mammary tumors. This can be interpreted as genistein not exerting a chemotherapeutic effect on existing tumors, and genistein not exacerbating development of previously existing mammary tumors.

2) On the other hand, dietary genistein to adult rats exposed prepubertally to genistein provides additional protection against mammary cancer. Prepubertal genistein exposure appears to "imprint for additional adult genistein chemoprevention.

REPORTABLE OUTCOMES

This data have been presented in part at the following meetings:

University of Missouri Sixth Annual Oncology Conference. Dietary Genistein Protects against Mammary and Prostate Cancers. Lake of the Ozarks, Missouri. April 27, 2001

FASEB Summer Research Conference on Physiological Functions of Antioxidant Nutrients and Phytochemicals. Genistein and Breast and Prostate Cancers. Tucson, AR. June 16-21, 2001

Hormonal Carcinogenesis Gordon Conference. Dietary Factors in Hormonal Carcinogenesis. Genistein Chemoprevention: Timing of Exposure and Mechanisms of Action (Mammary and Prostate). Kimball, NH, July 8-13, 2001.

CONCLUSIONS

We conclude that dietary genistein in adult life is only effective in protecting against chemically-induced mammary cancer if the female mammary gland has already been imprinted prepubertally.

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APPENDICES

Two figures

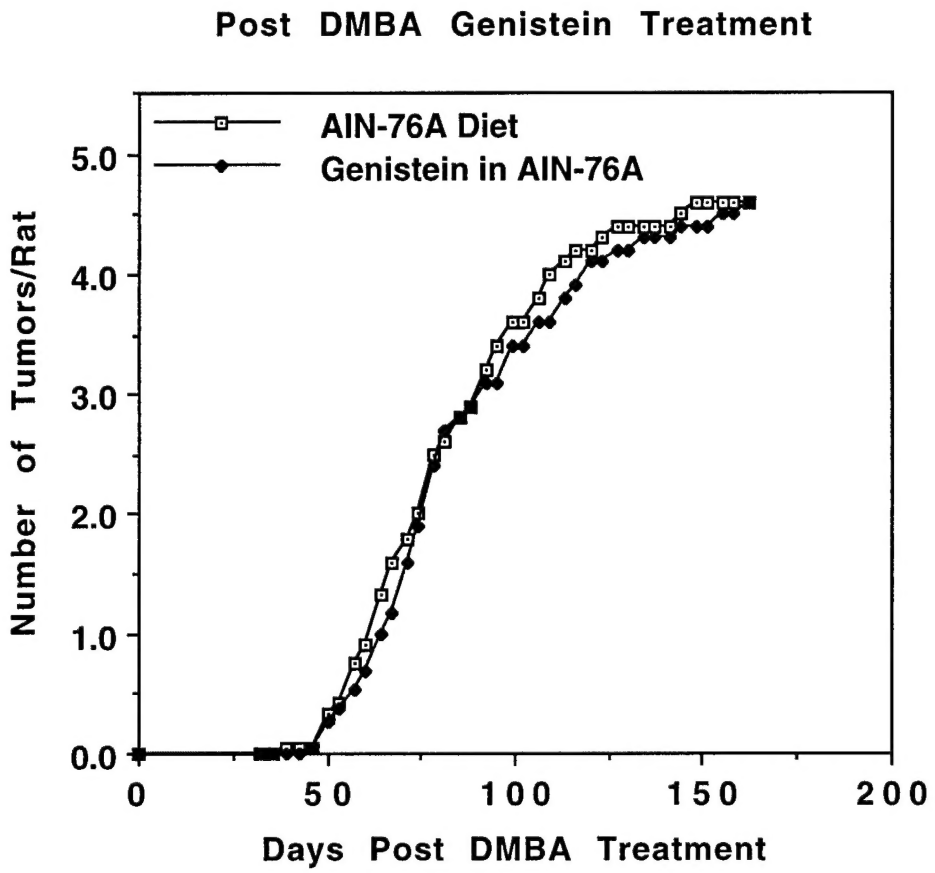

Figure 1. Adult dietary genistein effect on mammary tumors in rats exposed as adults to 80 mg DMBA/kg body weight at day 50 postpartum. One group was fed AIN-76A diet from parturition onward (AIN-76A Diet). The second group was fed AIN-76A diet from parturition through day 100 (50 days post-DMBA), then they were fed 250 mg genistein/kg AIN-76A diet (Genistein in AIN-76A).

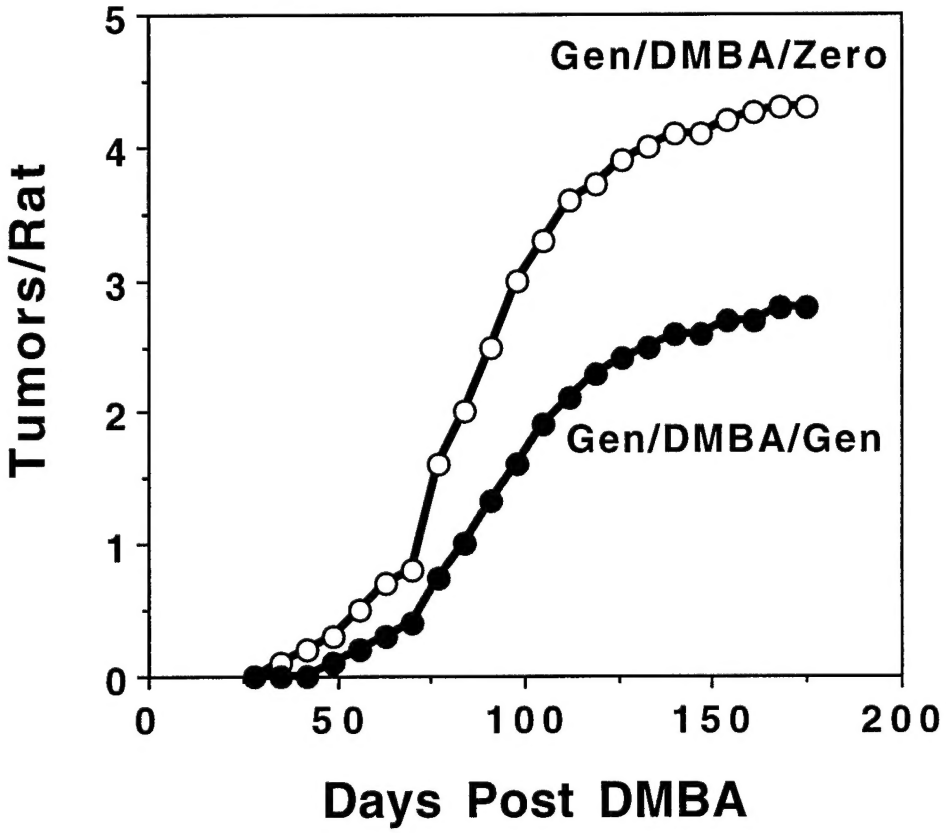

Figure 2. Adult dietary genistein effect on palpable mammary tumors in rats exposed prepubertally to genistein, and as adults to DMBA. One group was fed AIN-76A diet containing 250 mg genistein/kg diet, starting from parturition through day 21 only and then AIN-76A onward (Gen/DMBA/Zero). The second group was fed genistein containing diet from parturition through day 21, then AIN-76A only through day 100 postpartum and then from day 100, genistein containing diet (Gen/DMBA/Gen). All animals received 80 mg DMBA/kg body weight at day 50. Day 100 postpartum is 50 days after the DMBA was administered, shortly after the first tumors were palpable.